# Developing a new JE vaccine

## - Switch the mouse brain to the Vero cell -

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# Improvement of mouse brain-derived JE vaccines in Japan

1954 Developed,
using mouse brains

1965 Highly purified,
using alcohol, protamine sulfate,
by ultra-centrifugation

1988 Change of virus strain
from Nakayama to Beijing

# Disadvantage of using mouse brains

1. The vaccine may contain **adventitious viruses**.
   → difficulty in quality control

2. The vaccine may contain **brain ingredients**.
   → concerns about neurological adverse effects

3. We have to use **great number of mice**.
   → difficulties of production,
   against animal rights

4. And so on and so forth

# Vero cell bank

1. Vero cells were purchased from
   American Type Culture Collection (ATCC)

2. A cell bank system was established by
   making a master cell bank
   and working cell banks.

3. The Vero cells in the cell bank system cleared
   the Japanese and WHO standards
   for vaccine production.

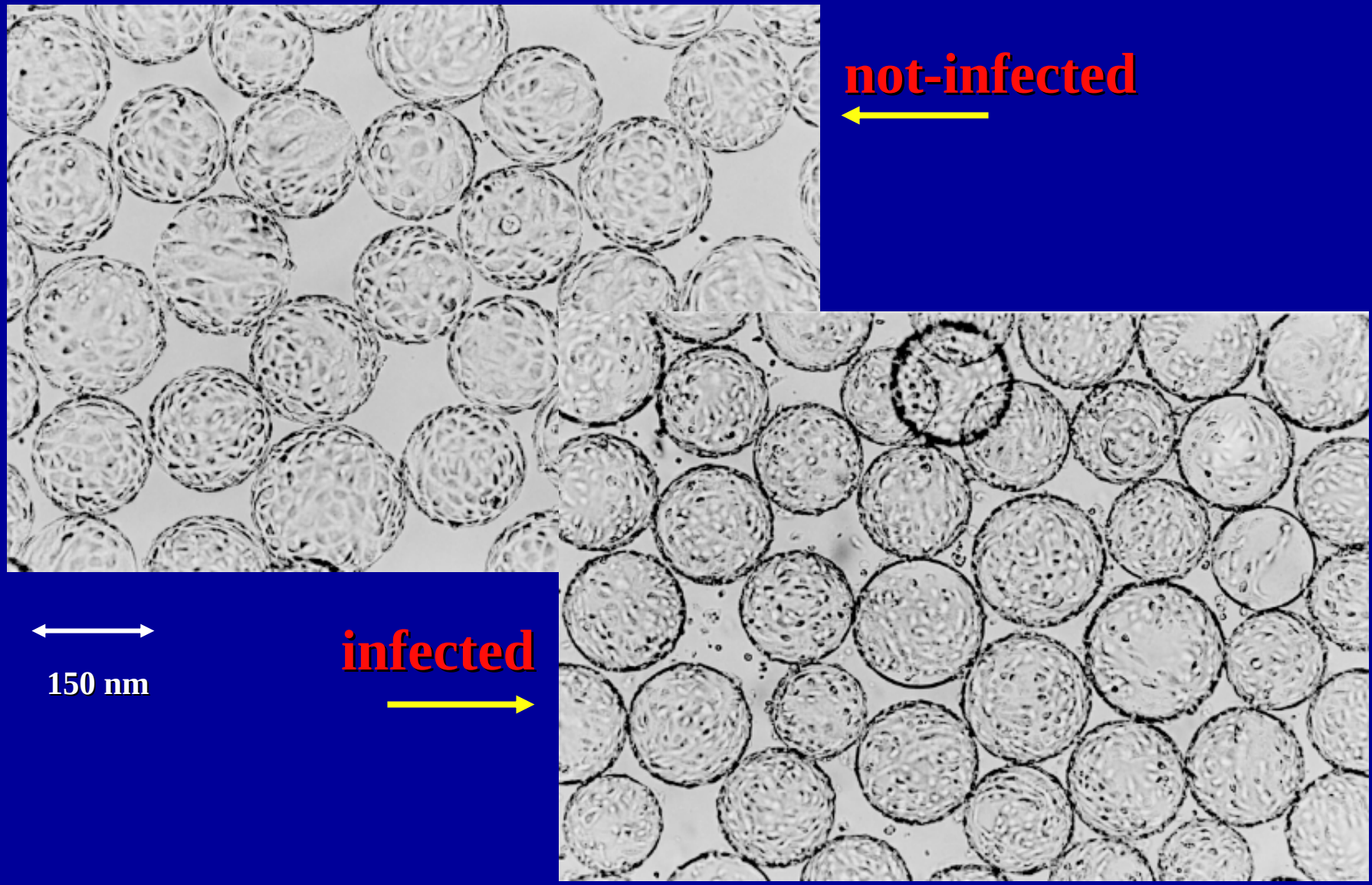
# The seed virus strain

The Beijing strain, the same strain used for production of the existing inactivated JE vaccine, was used for production of the new vaccine under a controlled condition of the Minimum Requirements for Biological Products of Japan (Seed Lot System).

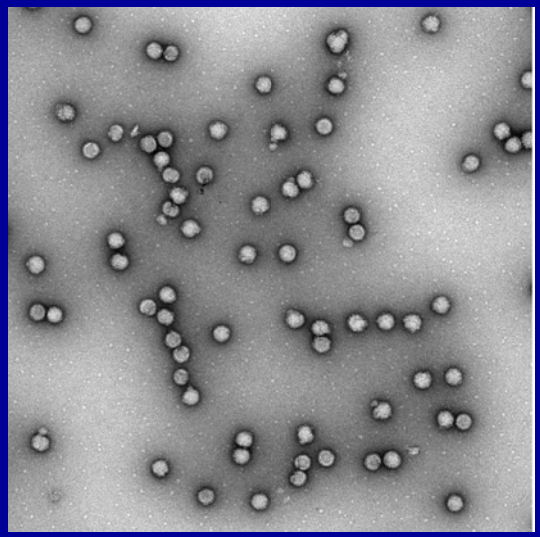
# Production process

1. Collection of culture fluid of Vero cells infected with the Beijing strain of JE virus.
2. Filtration of cell debris in the culture fluid
3. Inactivation with formalin
4. Purification with protamine sulfate, by ultra-centrifugation in sucrose density gradient
5. Dialysis of the virus fraction
6. Addition of stabilizers
7. Filling into vials
8. Freeze-drying
9. Filling with nitrogen gas

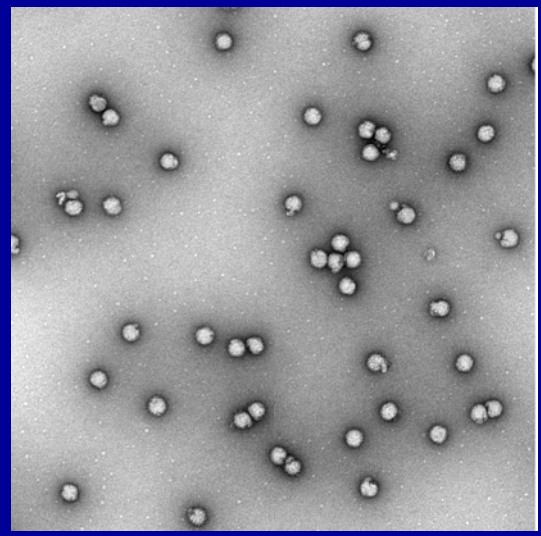
# Vero cell cultures on Cytodex 1



# Electron micrographs of the Vero cell-derived JE vaccine



BM-03001



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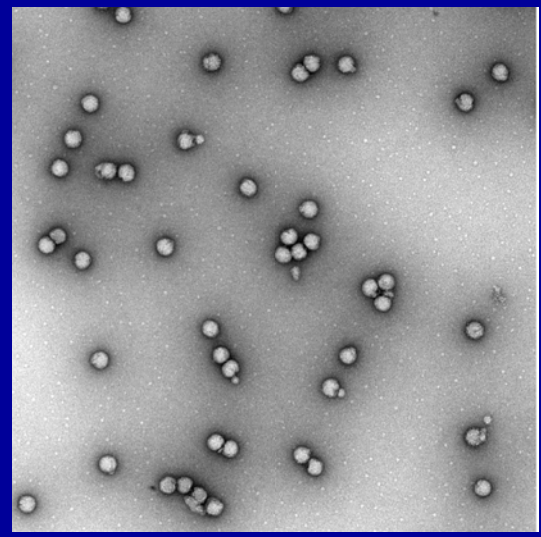

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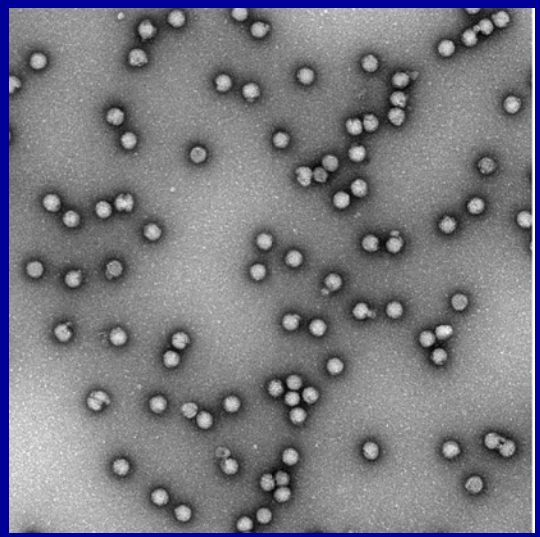
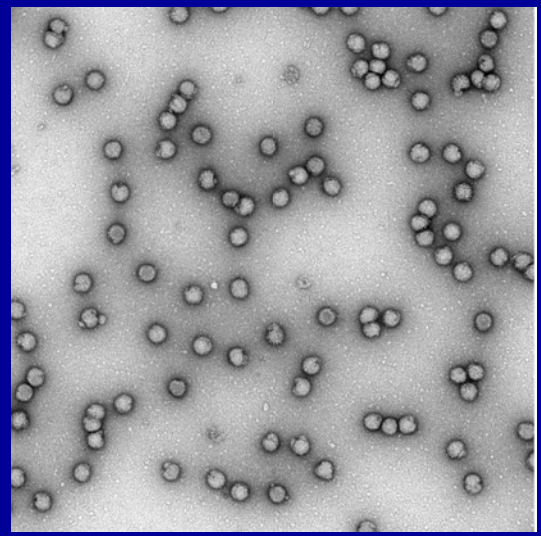
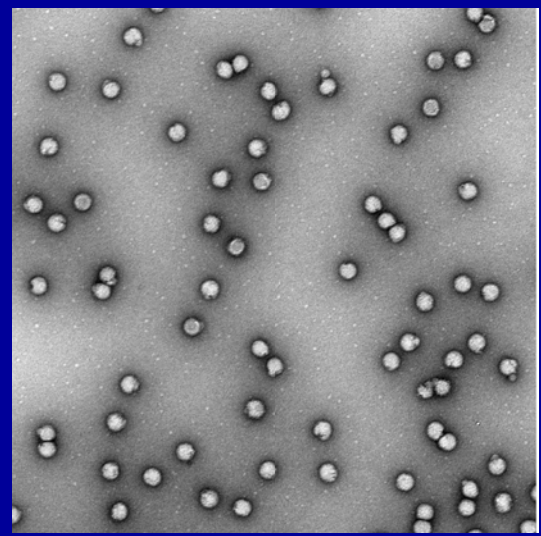


BM-99001/5 yrs



BM-99002/5 yrs



BM-99003/5 yrs

# Formulated products

| Component | BK-VJE Lyophilised | Existing vaccine Liquid |
|---|---|---|
| inactivated JE virus | more than Reference | |
| lactose | 17.86mg | |
| L-sodium glutamate | 3.57mg | |
| TCM-199 | 1.18mg | 5.5mg |
| formic aldehyde | 0.01mg | 0.1mg |
| D-sorbitol | | 5mg |
| polysorvate | | 0.0015mg |
| thimerosal | | 0.005mg |
| gelatin | | |
| term of validity | 5 years | 1 year |

# Specification of the Final Product

| Test item | BK-VJE | Existing vaccine |
|---|---|---|
| Sterility | No evidence of microbial growth | |
| Staining | No evidence of bacteria | |
| Moisture contents(%) | No higher than 3% . | |
| Inactivation | No abnormal sign during the observation period | |
| TCA-protein ( mcg/mL | No higher than 80mcg /ml | |
| Potency ( N Ab Titer in log) | No less than the Reference | |
| pH | The pH shall be within a range between 6.8 and 7.4 | |
| Residual mouse Serum(ng/dose) | None | .50 |
| Residual calf Serum (ng/dose) | .50 | None |
| Residual cellular DNA (pg/dose) | .50 | Not done |
| General safety Guinea pig, Mouse | None of the animals show any abnormal signs during the observation period | |

# Pre-Clinical Study

## (Safety Studies of BK-VJE vaccine)

1. Single-Dose Toxicity Study
2. Repeat-Dose Toxicity Study
3. Local Tolerance

→ The aberration by BK-VJE was not observed.

4. General Pharmacology Study
   1) Effects on excretion of urine and urinary electrolytes
   2) Effects on respiration rate and tidal volume

→ BK-VJE did not have any influence on urinary excretion and respiratory function.

5.Genetic Toxicity Test
   1)Bacterial Reversion Assay
   2)Gene Mutation Study:
      Using mouse lymphoma cells

→ The genetic toxicity was not detected in BK-VJE.

# Phase I Clinical Study (BK-VJE/001)

| | | |
|---|---|---|
| **Period** | : | **September November, 2001** |
| **Subjects** | : | **Healthy Japanese male adults** |
| **Number of subjects** | : | **BK-VJE ; 17 cases**<br>**Placebo(Saline) ; 3 cases** |
| **Dosage** | : | **2 doses at 14 days interval (s.c.)** |
| **Safety** | : | **Adverse events & laboratory data** |
| **Immunogenicity** | : | **Seroconversion rate & booster effect** |

# Adverse Reactions ( phase I clinical study)

| | Cases with Symptom | Degree | Onset | Therapy | Outcome | Outcome on |
|---|---|---|---|---|---|---|
| 1 | Redness at the site | mild | Day 1 | none | Resolved | Day 2 |
| 2 | AST | 57 IU/L** | Day 42 | none | Resolved | Day 57 |
| | ALT | 64 IU/L** | Day 42 | none | Resolved | Day 57 |
| | LDH | 222 IU/L** | Day 42 | none | Resolved | Day 57 |
| 3 | Redness at the site | mild | Day 1 | none | Resolved | Day 3 |
| 4 | Redness at the site | mild | Day 15 | none | Resolved | Day 3 |
| 5 | Redness at the site | mild | Day 1 | none | Resolved | Day 2 |

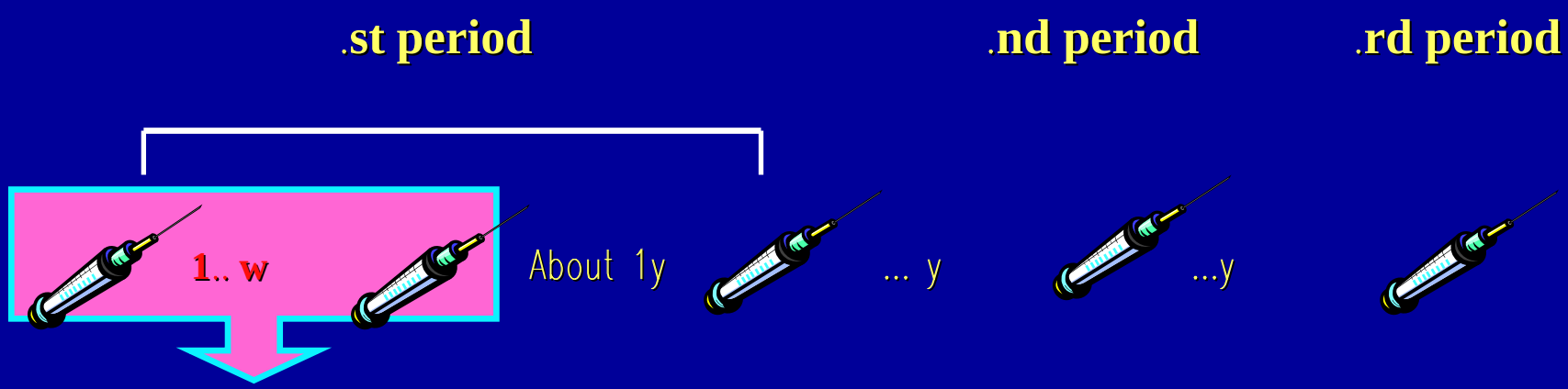
* : One day after the second injection

**: Normal value AST; 10-31 IU/L, ALT; 8-52 IU/L, LDH; 95-202 IU/L

# Immunogenicity: Neutralizing antibody response (Phase I Clinical Study)

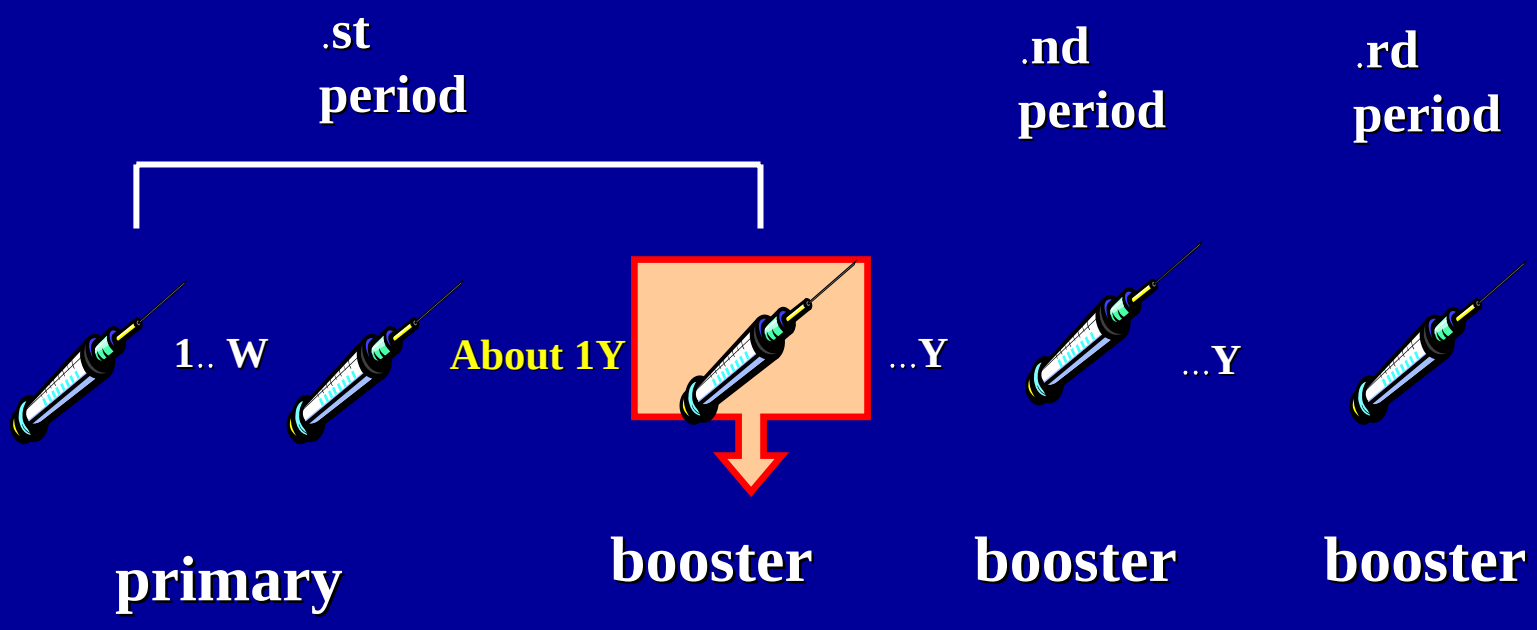
| Group | Pre | | Post | |
|---|---|---|---|---|
| | Antibody | Number | Sero conversion | Rise of antibody |
| BK-VJE | – (<20) | 7 | 7/7 (307) | . |
| | + (78) | 10 | . | 9/10 (518) |
| Placebo (Saline) | – (<20) | 1 | 0/1 (<20) | . |
| | + (100) | 2 | . | 0/2 (107) |

# Schedule of JE vaccination in Japan during the study




BK-VJE/002

# Schedule of JE vaccination in Japan during the study




BK-VJE/003

# Phase III Clinical Study (BK-VJE/002)

- Study Objective : Immunogenicity & Safety
- Study Design : Randomized, Single blind, Active control
- Subjects : Healthy children of 6-90 months
- Dose / Route : 2 Doses at 1-4 weeks interval (s.c.)
- Investigational vaccine : BK-VJE 116 cases

Control vaccine : Existing vaccine 109 cases

# Phase III Clinical Study (BK-VJE/003)

- Study Objective: Immunogenicity & Safety
- Study Design: Open labeled, Active control
- Subjects : Healthy children of 12-90 months
  (6-24 months after $2_{nd}$ dose in 002 trial)
- Investigational

  vaccine : BK-VJE 106 cases

  Control vaccine : Existing vaccine 89 cases

# Immunogenicity and Safety of BK-VJE
# -Summary of Phase III Clinical Studies-

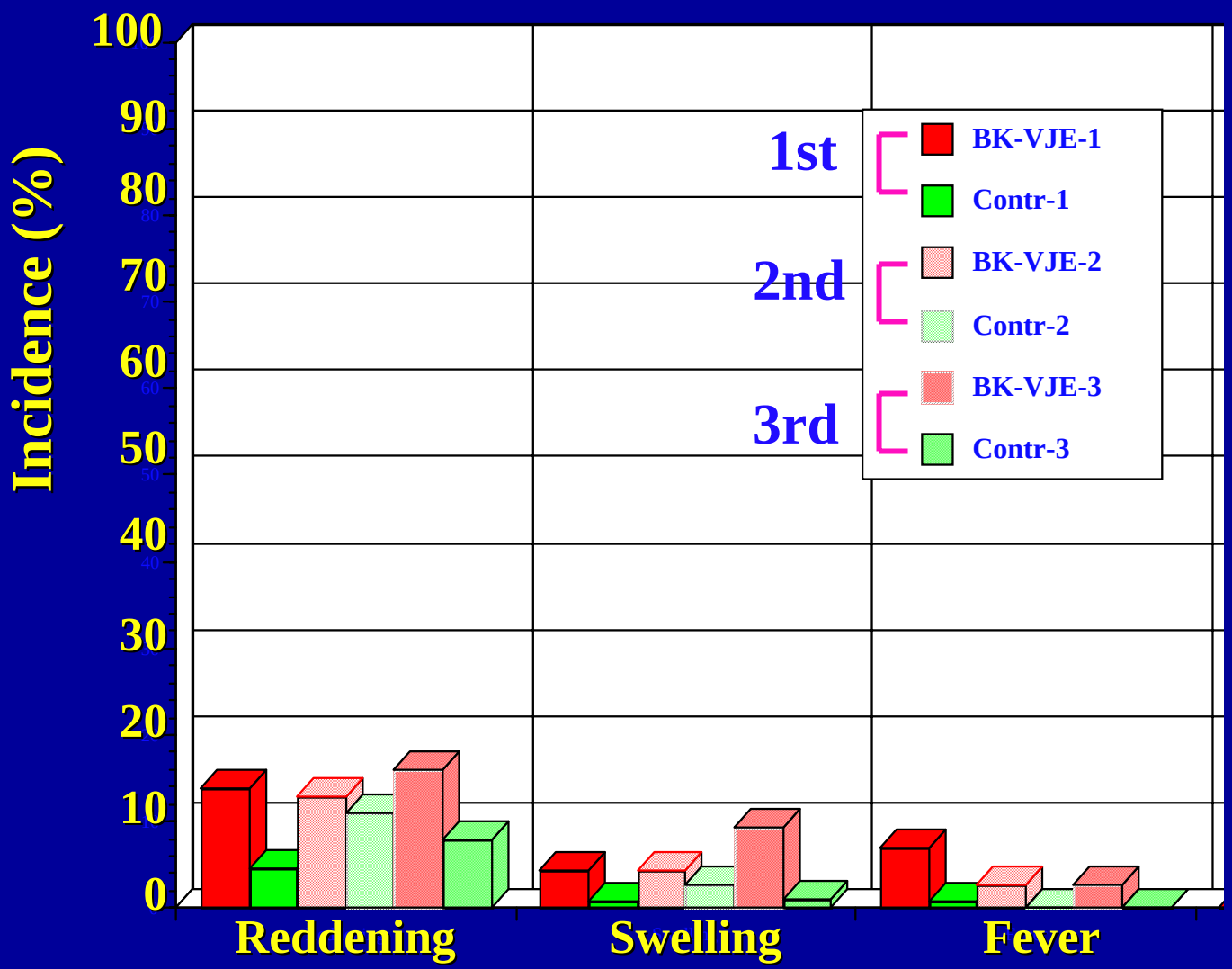
**Nt antibody titers** — **After the 1st 2 doses**

| | | |
|---|---|---|
| BK-VJE | 116 cases | 2.7 ($log_{10}$) |
| Control | 108 cases | 2.5 ($log_{10}$) |

| Nt antibody titers | | Before the booster | After the booster |
|---|---|---|---|
| BK-VJE | 106 cases | 2.6 ($log_{10}$) | 4.1 ($log_{10}$) |
| Control | 89 cases | 2.4 ($log_{10}$) | 3.9 ($log_{10}$) |

**Adverse reactions:** Only temporary
Reddening, Swelling at the injection site, and
Fever in some subjects

# Incidence of major adverse reactions



# *Next steps*

***We will continue clinical studies, the second-stage, with reduced amounts of the antigen in the vaccine.***


Thank you very much
for your attention!
I appreciate our colleagues
for developing the vaccine.
Shigeharu Ueda